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Aschersonia narathiwatensis sp. nov. from southern Thailand

SUCHADA MONGKOLSAMRIT[1]*, ARTIT KHONSANIT[1], WASANA NOISRIPOOM[1],
PAGMADULAM BALDORJ[2], & J. JENNIFER LUANGSA-ARD[1]

[1]*BIOTEC, NSTDA Science Park,*
113 Paholyothin Road, Klong 1, Klong Luang, Pathum Thani, Thailand
[2]*Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar-51, Mongolia*
* CORRESPONDENCE TO: *suchada@biotec.or.th*

ABSTRACT — *Aschersonia narathiwatensis* was isolated from whitefly nymphs (*Hemiptera*) collected at Hala-Bala Wildlife Sanctuary in Narathiwat Province, Thailand. This species has circular flattened-discoid brown to dark brown stroma and non-fragmenting ascospores. A comparison of macro-and microscopic characters among similar species is given. Phylogenetic analyses of nuclear ribosomal large subunit (LSU) and translation elongation factor 1-α (TEF-1α) support recognizing this fungus as a new species.

KEY WORDS — morphology, phylogenetics, taxonomy

Introduction

Aschersonia Mont. is a genus of entomopathogenic fungi that attack scale insects (*Coccidae, Hemiptera*) or white flies (*Aleyrodidae, Hemiptera*) of which several species are reported from tropical and subtropical regions (Montagne 1848; Petch 1921; Mains 1959a,b; Chaverri et al. 2008; Mongkolsamrit et al. 2009, 2011; Qiu et al. 2009, 2010; Qiu & Guan 2010). Research on entomopathogenic fungi has been conducted under the biodiversity studies program to further our knowledge of these fungi in Thailand. Here we describe a new *Aschersonia* species from a tropical rainforest in southern Thailand.

Materials & methods

Surveys and collections were made during the rainy season in Hala-Bala Wildlife Sanctuary, which is located at the southernmost tip of peninsular Thailand on the international border with Malaysia. Pure cultures were made from isolations from the teleomorphic state following Mongkolsamrit et al. (2009). Sections of the stroma prepared by using a freezing microtome were mounted in distilled water and in cotton blue in lactophenol (Heritage et al. 1996). Observation of the microscopic characters (e.g., perithecia, ascospores and asci) were made using a light microscope. The color of fresh specimens and cultures were compared with the colors from standard code of Kornerup & Wanscher (1962). A voucher specimen and culture were deposited in BIOTEC Bangkok Herbarium (BBH) and BIOTEC Culture Collection, Thailand.

Genomic DNA was extracted from each sample with cetyltrimethyl-ammonium bromide (CTAB) according to Mongkolsamrit et al. (2009). PCR amplification was done

Table 1. List of fungi used in molecular analyses.
(Newly generated sequences set in bold.)

Species	Voucher/Isolate	GenBank	
		LSU	EF1-α
Aschersonia badia	BBH 14255 = BCC 8105	DQ518752	**KC713630**
	BBH 32386 = BCC 55524	—	**KF016991**
A. calendulina	*BBH 17344 = BCC 20309	GU552154	**KF016993**
	BBH 17325 = BCC 20306	GU552148	**KF016994**
	*BCC 9483	DQ384938	DQ384962
A. luteola	*BBH 13011 = BCC 19360	GU552155	GU552147
	BBH 14277 = BCC 9481	DQ384945	DQ384974
	BBH 14078 = BCC 7865	DQ384946	DQ384975
A. minutispora	BBH 17373 = BCC 20635	GU552149	DQ552143
	BBH 14143 = BCC 7959	GU552150	—
	BBH 12951 = BCC 17487	GU552151	GU552144
A. narathiwatensis	*BBH 31120 = BCC 49495	**KC713633**	**KC713628**
	*BBH 31120 = BCC 49498	**KF016998**	—
	*BBH 31120 = BCC 49499	**KF016999**	**KF016992**
A. samoensis	BBH 14540 = BCC 8775	DQ384944	DQ384972
	*BCC 8237	DQ384937	DQ384977
	*NHJ 4093	AF327381	—
Balansia henningsiana	GAM 16112	AY489715	AY489610
Epichloe elymi	C. Schardl 760	AY986924	AY986951
Moelleriella phyllogena	P.C. 554 = CUP 067784	EU392609	EU392673
	P.C. 555 = CUP 067785	EU392610	EU392674
M. raciborskii	I93-901b = ARSEF 7661	EU392611	EU392676
M. umbospora	P.C. 457 = CUP 067816	AY986904	AY986929
Samuelsia geonomis	P.C. 614 = CUP 067857	EU392638	EU392692
S. mundiveteris	BBH 24706 = BCC 31750	GU552157	GU552142
	BBH 26961 = BCC 40021	GU552152	GU552145
	BBH 26961 = BCC 40022	GU552153	GU552146
S. rufobrunnea	P.C. 613 = CUP 067858	AY986918	AY986944

* Living cultures isolated from ascospores.

in 50 µl volumes consisting of 1× PCR buffer, 200 µM of each of the four dNTPs, 2.5 mM MgCl2, 1 U Taq DNA polymerase (Promega, Madison, Wisconsin), and 0.5 µM of each primer. The amplification of the partial gene region of the large subunit nuclear ribosomal DNA (LSU) used primers LRORf and LR5r (Vilgalys & Sun 1994) and the translation elongation factor 1-α (TEF-1α) was amplified with primers EF-1a: 983f (Carbone & Kohn 1999) and 2218r (Rehner 2001). After amplification in a MJ Research

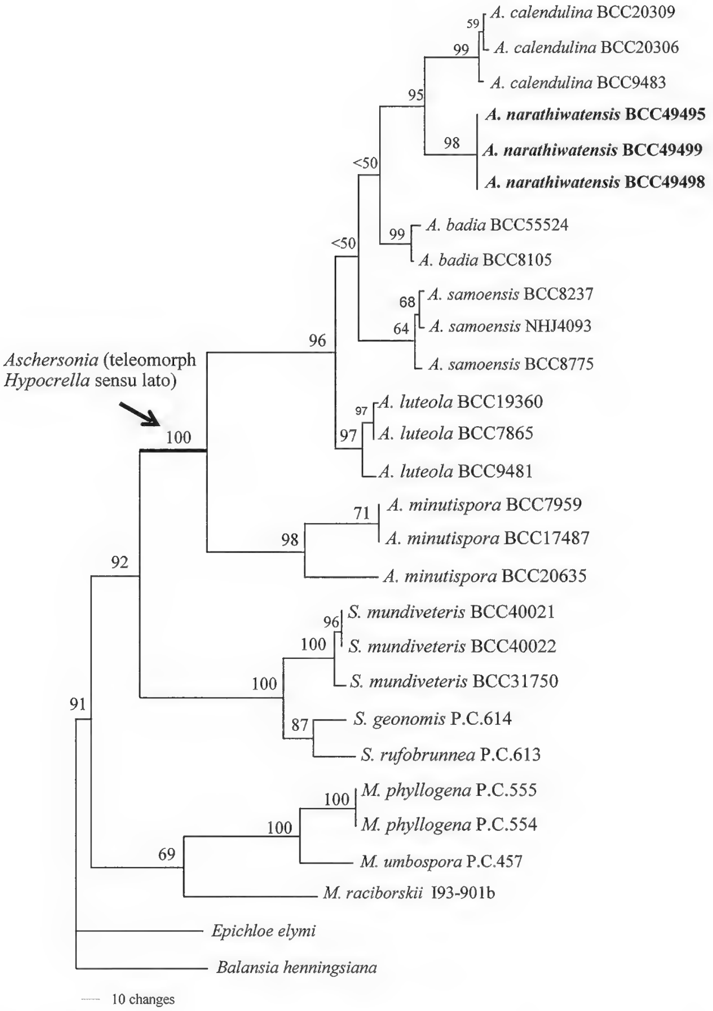

PLATE 1. Phylogenetic relationship of *Aschersonia narathiwatensis* and related species based on maximum parsimony analysis of the LSU and EF-1 α. Numbers above each branch represent bootstrap support from 1000 replicates. [*S.* = *Samuelsia; M.* = *Moelleriella*]

DNA Engine ALD1244 thermal cycler following the procedure described in Sung et al. (2001), the PCR products were purified with a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions. Purified PCR products were sent to Macrogen Inc. Korea for sequencing. Sequences were proofread manually and assembled using BioEdit v. 7.0.4 (Hall 1999), and deposited in GenBank (Table 1). Sequences were aligned with Clustal W incorporated in BioEdit, and alignments were refined manually by direct examination. Maximum parsimony analysis was performed on the combined dataset of LSU and *tef*-1α sequences in PAUP 4.0b10 using random addition sequence (10 replications) where gaps were treated as missing data. Bootstrap analysis was performed using maximum parsimony criterion in 1000 replication samples. *Balansia henningsiana* GAM 16112 and *Epichloe elymi* C. Schardl 760 were used as outgroup taxa.

Results

Molecular analysis

LSU sequences were obtained from three isolates, and *tef*-1α sequences from six isolates. Twenty-four LSU-related sequences and 19 TEF-1α-related sequences from GenBank were used to construct a phylogenetic tree. Of the 1652 characters in the combined alignment, 268 characters were parsimony informative. Maximum parsimony analyses of this data set yielded one parsimonious tree (tree length 698; CI = 0.610, RI = 0.797, RC = 0.486, HI = 0.390) as shown in Plate 1. The phylogenetic tree shows that three teleomorphic isolates assigned to our new species (BCC 49495, BCC 49498 and BCC 49499) belong to the genus *Aschersonia* with a strong bootstrap support of 100%.

Taxonomy

Aschersonia narathiwatensis Mongkols., Khonsanit & Luangsa-ard, **sp. nov.**

MycoBank MB 803933 Plate 2

Differs from *A. calendulina* by its brown to dark brown stromata and shorter ascospores.

Type — Thailand: Narathiwat Province, Headquarter Trail, Hala-Bala Wildlife Sanctuary, on whitefly nymphs (*Hemiptera*) on underside of dicotyledonous leaf, 24 Aug. 2011, A. Khonsanit (**Holotype**, BBH 31120; ex-holotype cultures BCC 49495 [GenBank, KC713633, KC713628], BCC 49498 [GenBank, KF016998], BCC 49499 [GenBank, KF016999, KF016992].

Etymology — referring to Narathiwat Province, the collection location.

Teleomorph: *Hypocrella*

Stromata circular, flattened-discoid, brown to dark brown, 5E7, ≤1–3 mm diam and 0.5–1 mm high, margin undulate, base slightly constricted; internally hyaline to whitish-coloured. Hypothallus membranous, slight or thickened, 0.5–1 mm thick, pale yellow. Perithecia crowded, immersed, 200–430 × 100–200 μm, elongate flask-shaped, ostioles slightly projecting, translucent

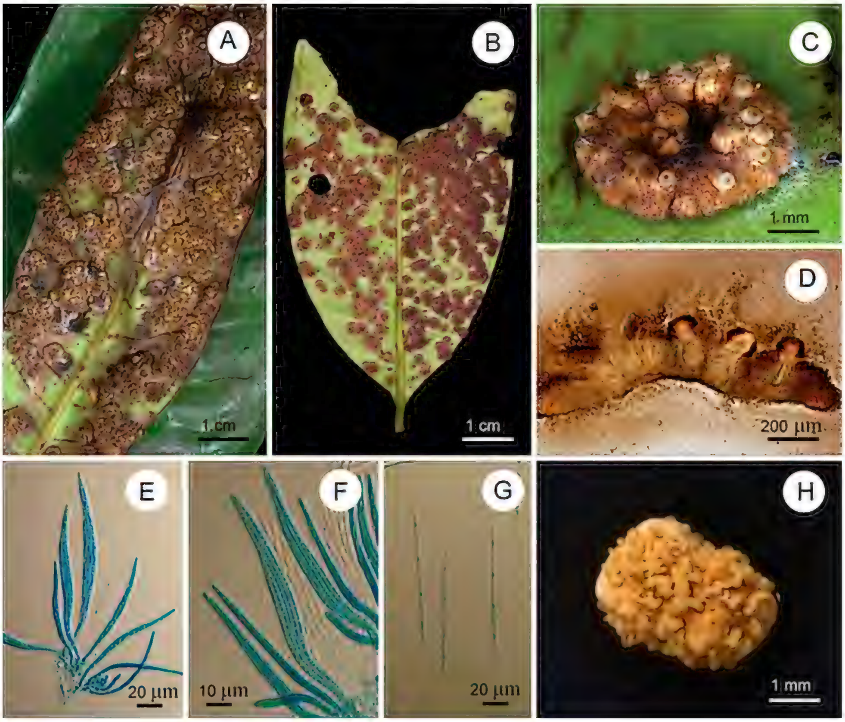

PLATE 2. *Aschersonia narathiwatensis*: A–C. Stromata on infected whitefly nymph hosts; D. Cross section of stroma showing perithecia; E, F. Mature ascus with developing asci; G. Whole ascospores; H. Sporulating colony on PDA at 20°C after 4 wk.

dark-brown. Asci 8-spored, ≤200 × 10–12 µm. Ascospores whole, non-fragmenting, 80–100 × 1.5–2 µm, blunt at upper end tapering to base.

CULTURAL CHARACTERISTICS Ascospores germinating within 24 h on PDA. Colonies on potato dextrose agar (PDA) slow-growing, attaining a diam of 5 mm in 4 wk. Optimal temperature 20–25°C, with no growth at <5°C and >35°C. Stromatic colonies pale brown, forming moderately compact stromata. Conidial masses pale brown, 4C6, appearing as abundant slimy masses from immersed pycnidia scattered over surface.

COMMENTS: In this study, the anamorphic generic name *Aschersonia* is used based on the recent major changes in fungal nomenclature requiring only one scientific name per fungal species (Hawksworth 2011; Taylor 2011; Gams et al. 2012; McNeill et al. 2012). Described earlier in 1848, *Aschersonia* has priority over the teleomorphic generic name, *Hypocrella* Sacc. (1878). In this study, *Hypocrella* is treated as the teleomorph of *Aschersonia*.

Aschersonia narathiwatensis was been found only at Hala-Bala Wildlife Sanctuary in southern Thailand. Despite several attempts to find it in different seasons of the year, the anamorphic state of this species was not found in the field. The teleomorphic characters are presented and compared with other Thai species representing *Hypocrella* sensu lato (Table 2). The teleomorphic state of *A. narathiwatensis* is similar to that of *A. calendulina* reported from Thailand by Mongkolsamrit et al. (2009) in having flattened discoid stromata but differs in the color and size of the stromata. *Aschersonia narathiwatensis* has brown to dark brown stromata while those of *A. calendulina* are bright orange. The ascospores of *A. narathiwatensis* are somewhat shorter than those of *A. calendulina* (Table 2). In the phylogenetic analysis, *A. narathiwatensis* and *A. calendulina* form a clade with 95% support. Both morphological characters and phylogenetic analysis of the partial gene regions of LSU and *tef*-1α support *A. narathiwatensis* as a new species.

Table 2. Morphological comparison of *Aschersonia* teleomorphs in Thailand.

Species	Stromatal shape	Stromatal colour	Asci (μm)	Ascospores (μm)	Reference
A. calendulina	Flattened discoid	Bright orange	<180 × 10–12	130–150 × 1.5–2	Mongkolsamrit et al. (2009)
A. luteola	Discoid to stud-shaped	Yellow	<170 × 7.5–8	75–120 × 2	Mongkolsamrit et al. (2009)
A. narathiwatensis	Flattened discoid	Brown	<200 × 10–12	80–100 × 1.5–2	This study
A. samoensis	Discoid to stud-shaped	Rust orange	140–180 × 8	60–90 × 4–5	Hywel-Jones & Evans (1993)

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